Attorney Docket: RNBO-1-1003
Title: METHODS AND COMPOSITIONS FOR
TREATING MICROTUBULE-MEDIATED VIRAL
INFECTIONS AND LESIONS
Serial No. --Filing Date: March 5, 2002

Page 1 of 11

FIGURE 1A. Drug Screening Report for TBT, Lot ARB-ID#99-332

VEHICLE	634 д	g/ml in EtOH			UNIT mg						
LOT #1		TRIA	AL #1								
HSV-1 (HFF Cells)											
CPE Inhibition – MCG	ML										
09/17/98	EC50 >1	0 CC5	0 >10	SI = 0	ACV EC50 = 1.2						
Plaque Reduction – Mo	CG/ML										
10/19/98	EC50 = 5	.9 CC5	0 >10	SI >1.6	ACV EC50 = 0.3						
HSV-2 (HFF Cells)											
CPE Inhibition – MCG/ML											
09/17/98	EC50 = 2	.0 CC5	0 >10	SI >6.0	ACV EC50 = 1.9						
Plaque Reduction – MCG/ML											
10/19/98 EC50 = 0.9 CC50 > 10 SI > 11.1 ACV EC50 = 0.1											
HCMV (HFF Cells)											
CPE Inhibition – MCG	ML										
10/01/98	EC50 = 5	.0 CC5	0 >10	SI >2.0	GCV EC50 = 0.06						
Plaque Reduction – Mo	CG/ML										
/ /	EC50	CC	C50	SI	GCV EC50						
MCMV (HFF Cells)											
Plaque Reduction - M	CG/ML										
//	EC50	CC	C50	SI	CCV EC50						
VZV (HFF Cells)											
CPE Inhibition – MCG	i/ML										
/ /	EC50	CC	C50	SI	ACV EC50						
Plaque Reduction – M	CG/ML										
11	CC	C50	SI	CCV EC50							

Cytopathic Effect Inhibition and Plaque Reduction Assays For HSV-1, HSV-2, HCMV, MCMV, and VSV, in HFF cell cultures.

Attorney Docket: RNBO-1-1003
Title: METHODS AND COMPOSITIONS FOR
TREATING MICROTUBULE-MEDIATED VIRAL
INFECTIONS AND LESIONS
Serial No. --Filing Date: March 5, 2002
Page 2of 11

FIGURE 1B. Drug Screening Report for TBT, Lot ARB-ID#99-332

EBV										
VCA – MCG/ML (In Daudi Cells)										
10/16/98	EC50 = 1.4	0 = 1.4 $CC50 = 0.21$ $SI = 0$ $ACV EC50 = 1.8$								
EBV DNA – MCG/ML (In Daudi Cells)										
// EC50 CC50 SI ACV EC50										
Toxicity Assays										
Neutral Red Uptake –	Neutral Red Uptake – MCG/ML (In Stationary HFF Cells)									
09/17/98	CC5)>10	ACV	IC50 >	100		GCV IC50 >100			
Cell Proliferation – M	ICG/ML (In Rapidl	y Growing Dat	udi Cells)							
10/16/98	;	IC50	<0.08			ACV	IC50 >50			
Cell Proliferation – MCG/ML (In Rapidly Growing HFF Cells)										
10/16/98	IC50	0.02	ACV	IC50 >	100		GCV IC50 40.0			
Comments and Recon	Comments and Recommendations: HCMV PR. This compound is problematic.									

Cytopathic Effect Inhibition and Plaque Reduction Assays EBV in DAUDI cell cultures; Toxicity Assays in HFF and DAUDI cell cultures.

Attorney Docket: RNBO-1-1003

Title: METHODS AND COMPOSITIONS FOR TREATING MICROTUBULE-MEDIATED VIRAL

INFECTIONS AND LESIONS

Serial No. ---

Filing Date: March 5, 2002

Page 3 of 11

FIGURE 2. HSV ELISA METHOD

- 1. Remove the growth media HFF cells.
- 2. Add $100 \mu l$ of MEM to the 6 cell control wells.
- 3. Add 50 µl of the diluted virus to the remaining wells.
 - A. Herpes simplex virus type 1, ATCC # V-733, strain F. HSV-1 (5/3/96), one plate. Dilute stock 1:2000, 2.0 μl in 4.0 ml MEM.
 - B. Herpes simplex virus type 2, ATCC # VR-734, strain G. HSV-2 (5/3/96), one plate. Dilute stock 1:2000, $2.0 \mu l + 4.0 ml$ MEM.

Clinical strains.

- 1. Specimen # 1. HSV-1, December 9, 1997, eye. Dilute stock 1:500, 8.0 μl plus 4.0 ml of MEM.
- 2. Specimen # 2, HSV-1, September 30, 1997, oral pharynx. Dilute stock 1:50, 60 µl plus 3.0 ml of MEM.
- 3. Specimen # 3, HSV-2, Nov. 26, 96, oral pharynx. Dilute stock 1:500, 8.0 µl plus 4.0 ml of MEM.
- 4. Specimen # 4, HSV-2, Dec. 27, 96, ETT. Dilute stock (May 27, 98) 1:100, 40.0 µl plus 4.0 ml of MEM.
- 5. Specimen # 5. HSV-2, April 11, 1998, penis. Dilute stock 1:100, 40 μl plus 4.0 ml of MEM.
- 6. Specimen # 6. HSV-2, April 24, 1998. Dilute stock 1:100, 40 µl plus 4.0 ml of MEM.
- 4. Add the virus to the plates for one hour and incubate the plates at 37° C.
- 5. Remove the virus inoculum and add 100 μ l of MEM to the virus control wells and add 100 μ l of diluted drug to each of the remaining wells.

TBT. Stock TBT contains 624 μ g/ml of active component, 10-deacetyl-7-xylosyltaxol in 50% ethanol from Rainbow Research, Inc.

a. Warm the TBT and 1X MEM to 37° C. The stock TBT was diluted to 4.0 μ g/ml by adding 154 μ l of TBT to 24,000 μ l of 1X MEM.

TBT, μg/ml	4.0	3.0	2.0	1.0	0.75	0.5	0.25	0.1
Vol. Of extract, µl.	6000	6000	4000	2000	1500	1000	500	200
МЕМ, μl.	ı	2000	4000	6000	6500	7000	7500	7800

ELISA RESULTS:

Antibody to HSV-1 was used at a 1:2000 dilution. Antibody to HSV-2 was used at a 1:1500 dilution. Read with dual wavelength, 630 – 490 nm.

Attorney Docket: RNBO-1-1003
Title: METHODS AND COMPOSITIONS FOR
TREATING MICROTUBULE-MEDIATED VIRAL
INFECTIONS AND LESIONS

Serial No. ---

Filing Date: March 5, 2002

Page 4 of 11

FIGURE 3

HSV-1 ELISA CONTROL (ATCC VR-733, STRAIN F)

CC	VC	4	3	2	1	0.75	0.5	0.25	0.1	μg/ml
0.000	0.361	0.069	0.129	0.188	0.373	0.360	0.367	0.309	0.305	
0.000	0.231	0.046	0.093	0.236	0.349	0.357	0.329	0.352	0.362	
0.000	0.304	0.052	0.114	0.206	0.358	0.327	0.291	0.355	0.354	
0.000	0.295	0.033	0.097	0.247	0.338	0.354	0.306	0.316	0.381	
0.000	0.290	0.039	0.098	0.241	0.312	0.301	0.347	0.346	0.361	
0.000	0.303	0.045	0.065	0.203	0.344	0.349	0.333	0.362	0.346	
0.000	0.297	0.047	0.099	0.220	0.346	0.341	0.329	0.340	0.352	
	0.297	0.047	0.099	0.220	0.346	0.341	0.329	0.340	0.352	
		15.9	33.4	74.0	116.3	114.8	110.6	114.3	118.2	
DATE	: 6/24/9	98			INOC:	6/22/9	8			
DRUG: TBT					VIRUS: HSV-1					
					Control TBT-IC50 = $3 \mu g/ml$					

Attorney Docket: RNBO-1-1003
Title: METHODS AND COMPOSITIONS FOR
TREATING MICROTUBULE-MEDIATED VIRAL
INFECTIONS AND LESIONS

Serial No. ---Filing Date: March 5, 2002 Page 5 of 11

FIGURE 4 HSV-1 ELISA, CLINICAL SPECIMEN #1

CC	VC	4	3	2	1	0.75	0.5	0.25	0.1	μg/ml
0.000	0.387	0.147	0.155	0.157	0.331	0.256	0.319	0.272	0.368	
0.000	0.279	0.107	0.166	0.161	0.273	0.235	0.258	0.238	0.370	
0.002	0.256	0.109	0.115	0.203	0.289	0.187	0.258	0.245	0.368	
0.000	0.328	0.110	0.135	0.216	0.295	0.251	0.231	0.274	0.370	
0.005	0.328	0.102	0.142	0.249	0.327	0.318	0.294	0.366	0.314	
0.003	0.344	0.098	0.198	0.204	0.316	0.311	0.324	0.374	0.362	
0.002	0.320	0.112	0.152	0.198	0.305	0.260	0.281	0.295	0.359	
	0.319	0.111	0.150	0.197	0.304	0.258	0.279	0.293	0.357	
										,
		34.7	47.1	61.7	95.2	81.0	87.6	92.0	112.0	
DATE	: 6/24/9	8			INOC:	6/22/9	8			
DRUC	: TBT				VIRUS: HSV-1					
					Specimen #1 TBT-IC50 = 3 µg/ml					

Column headings for the ELISA are culture control (CC) representing uninfected cell culture; Viral Control (VC), representing infected cell culture; 4-3-2-1-0.75-0.5-0.25-0.1

are viral infected cell cultures to which 4 to $0.1~\mu g/ml$ Taxus Brevifolia Tincture (TBT) has been added.

Attorney Docket: RNBO-1-1003 Title: METHODS AND COMPOSITIONS FOR TREATING MICROTUBULE-MEDIATED VIRAL INFECTIONS AND LESIONS

Serial No. ---

Filing Date: March 5, 2002

Page 6 of 11

FIGURE 5 HSV-1 ELISA, CLINICAL SPECIMEN #2

CC	VC	4	3	2	1	0.75	0.5	0.25	0.1	μg/ml	
0.005	0.238	0.016	0.005	0.020	0.048	0.061	0.029	0.029	0.062		
0.002	0.086	0.005	0.019	0.050	0.053	0.054	0.080	0.080	0.056		
0.009	0.111	0.010	0.000	0.000	0.033	0.027	0.104	0.104	0.077		
0.006	0.141	0.034	0.011	0.022	0.065	0.059	0.059	0.059	0.109		
0.012	0.186	0.002	0.057	0.110	0.061	0.087	0.108	0.108	0.086		
0.006	0.196	0.018	0.039	0.017	0.031	0.087	0.090	0.090	0.120		
0.007	0.160	0.014	0.022	0.037	0.049	0.063	0.078	0.078	0.085		
					·						
	0.153	0.008	0.015	0.030	0.042	0.056	0.072	0.072	0.078		
		4.9	9.9	19.5	27.3	36.5	46.8	46.8	51.2		
DATE	: 6/24/9	98			INOC:	6/22/9	8				
DRUC	DRUG: TBT					VIRUS: HSV-1					
						Specimen #2 TBT-IC50 = 0.25 µg/ml					

Attorney Docket: RNBO-1-1003
Title: METHODS AND COMPOSITIONS FOR
TREATING MICROTUBULE-MEDIATED VIRAL
INFECTIONS AND LESIONS
Serial No. --Filing Date: March 5, 2002
Page 7 of 11

FIGURE 6 HSV-2 ELISA CONTROL (ATCC VR-734, STRAIN G)

CC	VC	4	3	2	1	0.75	0.5	0.25	0.1	μg/ml	
0.007	0.172	0.019	0.053	0.033	0.098	0.065	0.088	0.179	0.244		
0.007	0.148	0.016	0.049	0.074	0.079	0.108	0.125	0.150	0.201		
0.005	0.147	0.024	0.014	0.117	0.066	0.086	0.079	0.113	0.245		
0.015	0.169	0.044	0.033	0.060	0.070	0.097	0.107	0.124	0.203		
0.029	0.231	0.026	0.045	0.073	0.068	0.080	0.108	0.155	0.223		
0.008	0.148	0.015	0.031	0.053	0.067	0.103	0.085	0.104	0.177		
0.012	0.169	0.024	0.038	0.068	0.075	0.090	0.099	0.138	0.216		
	0.157	0.012	0.026	0.057	0.063	0.078	0.087	0.126	0.204		
		7.7	16.3	35.9	39.9	49.6	55.2	79.9	129.4		
DATE	: 6/24/9	8			INOC: 6/22/98						
DRUG	DRUG: TBT					VIRUS: HSV-2					
					Control TBT-IC50 = 0.75 µg/ml						

Attorney Docket: RNBO-1-1003 Title: METHODS AND COMPOSITIONS FOR TREATING MICROTUBULE-MEDIATED VIRAL INFECTIONS AND LESIONS Serial No. ---Filing Date: March 5, 2002

Page 8 of 11

FIGURE 7 HSV-2 ELISA, CLINICAL SPECIMEN #3

CC	VC	4	3	2	1	0.75	0.5	0.25	0.1	μg/ml	
0.015	0.337	0.119	0.109	0.145	0.290	0.137	0.200	0.264	0.204		
0.008	0.266	0.109	0.105	0.168	0.167	0.129	0.124	0.249	0.248		
0.010	0.207	0.056	0.077	0.154	0.215	0.106	0.158	0.191	0.253		
0.015	0.213	0.079	0.134	0.141	0.171	0.155	0.288	0.183	0.165		
0.027	0.211	0.131	0.090	0.105	0.171	0.122	0.203	.0189	0.168		
0.007	0.217	0.070	0.080	0.059	0.139	0.132	0.110	0.125	0.145		
0.014	0.242	0.094	0.099	0.129	0.192	0.130	0.181	0.200	0.197		
	0.228	0.080	0.086	0.115	0.179	0.117	0.167	0.187	0.184		
-											
		35.2	37.5	50.4	78.2	51.1	73.1	81.7	80.4		
DATE: 6/24/98					INOC:	6/22/9	8				
DRUG	DRUG: TBT					VIRUS: HSV-2					
					Specimen #3 TBT-IC50 = 2.0 µg/ml						

Attorney Docket: RNBO-1-1003
Title: METHODS AND COMPOSITIONS FOR
TREATING MICROTUBULE-MEDIATED VIRAL

INFECTIONS AND LESIONS Serial No. ---

Filing Date: March 5, 2002

Page 9 of 11

FIGURE 8 HSV-2 ELISA, CLINICAL SPECIMEN # 4

CC	VC	4	3	2	1	0.75	0.5	0.25	0.1	μg/ml
0.012	0.190	0.026	0.021	0.059	0.089	0.018	0.066	0.115	0.148	10
0.004	0.152	0.018	0.028	0.037	0.059	0.072	0.083	0.106	0.165	
0.030	0.199	0.001	0.042	0.033	0.026	0.052	0.065	0.073	0.195	
0.012	0.174	0.016	0.042	0.046	0.095	0.028	0.064	0.123	0.161	
0.020	0.224	0.033	0.048	0.027	0.031	0.025	0.100	0.078	0.229	
0.010	0.213	0.021	0.025	0.029	0.056	0.046	0.086	0.088	0.152	
0.015	0.192	0.019	0.034	0.039	0.059	0.040	0.077	0.097	0.175	
	0.177	0.005	0.020	0.024	0.045	0.026	0.063	0.083	0.160	
		2.5	11.1	13.4	25.2	14.4	35.3	46.5	90.4	
DATE	: 6/24/9	8			INOC:	6/22/9	8			
DRUG	: TBT				VIRUS: HSV-2					
Specimen #4 TBT-IC50 = 0								250 = 0.2	25 μg/m	1

Attorney Docket: RNBO-1-1003
Title: METHODS AND COMPOSITIONS FOR
TREATING MICROTUBULE-MEDIATED VIRAL
INFECTIONS AND LESIONS
Serial No. --Filing Date: March 5, 2002
Page 10 of 11

FIGURE 9 HSV-2 ELISA, CLINICAL SPECIMEN #5

CC	VC	4	3	2	1	0.75	0.5	0.25	0.1	μg/ml	
0.016	0.220	0.041	0.026	0.052	0.121	0.134	0.109	0.159	0.104		
0.013	0.219	0.017	0.045	0.048	0.084	0.078	0.084	0.130	0.114	-	
0.003	0.128	0.031	0.067	0.029	0.069	0.071	0.043	0.107	0.120		
0.033	0.210	0.010	0.051	0.077	0.061	0.062	0.055	0.184	0.100		
0.022	0.256	0.025	0.068	0.094	0.062	0.083	0.104	0.131	0.200		
0.036	0.167	0.018	0.083	0.137	0.131	0.111	0.107	0.134	0.141		
				,							
0.021	0.200	0.024	0.057	0.073	0.088	0.090	0.084	0.141	0.130		
	0.180	0.003	0.036	0.052	0.068	0.069	0.063	0.120	0.109		
		1.8	20.1	29.2	37.6	38.6	35.2	67.0	60.9		
DATE	: 6/24/9	8			INOC: 6/22/98						
DRUG: TBT					VIRUS: HSV-2						
					Specin	nen #5	TBT-IC	250 = 0.3	5 μg/ml		

Attorney Docket: RNBO-1-1003

Title: METHODS AND COMPOSITIONS FOR TREATING MICROTUBULE-MEDIATED VIRAL

INFECTIONS AND LESIONS

Serial No. ---

Filing Date: March 5, 2002

Page 11 of 11

FIGURE 10

HSV-2 ELISA, CLINICAL SPECIMEN #6

CC	VC	4	3	2	1	0.75	0.5	0.25	0.1	μg/ml
0.013	0.125	0.024	0.022	0.015	0.014	0.045	0.018	0.013	0.039	
0.012	0.154	0.028	0.017	0.111	0.011	0.077	0.037	0.055	0.050	
0.019	0.146	0.015	0.061	0.061	0.019	0.009	0.009	0.079	0.103	
0.017	0.128	0.043	0.021	0.024	0.124	0.012	0.037	0.058	0.075	
0.017	0.128	0.045	0.024	0.023	0.102	0.032	0.058	0.061	0.049	
0.033	0.092	0.029	0.017	0.033	0.035	0.061	0.021	0.082	0.077	
0.019	0.129	0.031	0.027	0.045	0.051	0.039	0.030	0.058	0.066	
	0.110	0.012	0.009	0.026	0.032	0.021	0.012	0.040	0.047	
		11.0	7.7	23.6	29.3	18.9	10.4	35.8	42.6	
DATE	: 6/24/9	8			INOC:	6/22/9	8			
DRUG	: TBT			9.4	VIRUS: HSV-2					
					Specimen #6 TBT-IC50 = 0.1 µg/ml					